HHS Public Access

Author manuscript

Pediatr Allergy Immunol. Author manuscript; available in PMC 2019 December 01.

Published in final edited form as:

Pediatr Allergy Immunol. 2018 December ; 29(8): 877–880. doi:10.1111/pai.12977.

Circulating 25-hydroxyvitamin D, nasopharyngeal microbiota, and bronchiolitis severity

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Keywords

airway microbiota; children; infant; microbiome; respiratory infection; vitamin D

To the Editor,

Bronchiolitis is the leading cause of hospitalization in U.S. infants, among which approximately 15% require intensive care.[1] Emerging evidence suggest a complex interplay between viral infection, airway microbiota, and host immune response in the pathobiology of bronchiolitis.[2] Vitamin D compounds have pleiotropic regulatory roles on both innate and adaptive immune responses.[3] Low levels of 25-hydroxyvitamin D (25OHD), the major circulating form of vitamin D, have been found to be associated with higher risk and severity of acute respiratory infections (ARIs) in children.[4] Recent studies also have reported an association of maternal[5] and cord blood[6] vitamin D levels with infant gut microbiota. Yet, there have been no studies investigating interactions between circulating 25OHD and airway microbiota on ARI severity in children. We have previously reported that infants with *Haemophilus*-dominant nasopharyngeal microbiota profile are at highest risk for intensive care use during bronchiolitis hospitalization.[1] In the current analysis, we examined the same

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Author contributions:

L.T. carried out the statistical analysis, drafted the initial manuscript, and approved the final manuscript as submitted. K.H. conceptualized and designed the study, carried out the statistical analysis, reviewed and revised the initial manuscript, and approved the final manuscript as submitted. N.J.A. and J.F.P. generated the microbiome data, carried out the initial statistical analysis, reviewed and revised the manuscript, and approved the final manuscript as submitted. JCC collected cohort data, reviewed and revised the manuscript, and approved the final manuscript as submitted. J.M.M. and C.A.C. conceptualized and designed the study, obtained the funding, supervised the conduct of the study, reviewed and revised the manuscript, and approved the final manuscript as submitted.

Conflicts of interest: The authors have no conflicts of interest relevant to this article to disclose.

cohort of infants with bronchiolitis to investigate the interactions between serum 25OHD and the nasopharyngeal microbiota with regard to bronchiolitis severity.

This is an analysis of the data from the 35th Multicenter Airway Research Collaboration (MARC-35) cohort study, a multicenter prospective cohort study of infants hospitalized for bronchiolitis (i.e., severe bronchiolitis). The details of the study design and analysis may be found in the Online Supplement. Briefly, 17 sites across 14 U.S. states enrolled 1,016 infants (aged <1 year) hospitalized for bronchiolitis (Table E1). Bronchiolitis was defined according to the American Academy of Pediatrics guidelines.[7] The institutional review boards at all participating sites approved the study. Informed consent was obtained from the infants' parent or legal guardian.

Background and clinical data were collected via structured interview and chart reviews. Serum and nasopharyngeal airway samples were collected within 24 hours of hospitalization using standardized protocols.[1] Serum total 25OHD levels were quantified by immunoassays. Nasopharyngeal samples were analyzed for microbiota using 16S rRNA gene sequencing. Nasopharyngeal microbiota profiles were derived by using partitioning around medoids (PAM) unbiased clustering with the use of weighted UniFrac distance.[1]

For the current analysis, the total 25OHD levels were dichotomized based on the median level into lower (<26.5 ng/ml) and higher (≥26.5 ng/ml) groups. The primary outcome was intensive care use, defined as intensive care unit admission or use of mechanical ventilation (continuous positive airway pressure or intubation) during bronchiolitis hospitalization. To test for a statistical interaction between serum total 25OHD and nasopharyngeal microbiota profiles – with regard to intensive care use – random-effects models were constructed accounting for the between-hospital differences (e.g., the differences in intensive care use) and adjusting for 12 patient-level covariates. As the models indicated a significant interaction, the analysis was then stratified by total 25OHD status. Data were analyzed using R version 3.4.4.

Of 1,016 infants with bronchiolitis, 1,005 (99%) met the quality control requirements for 16S rRNA gene sequencing. The median age was 3.2 months (IQR 1.6–5.9) and 60% were male. The median serum total 25OHD level was 26.5 ng/ml (IQR 18.0–33.1 ng/ml). Altogether 161 (16%) infants had intensive care use during a bronchiolitis hospitalization. The baseline characteristics of infants by 25OHD status are shown in Table E2. Compared with infants with higher 25OHD levels, those with lower 25OHD levels were younger and more likely to have household siblings and breastfeeding, but less likely to have a history of breathing problems and antibiotic use (all P<0.05).

In the nasopharyngeal samples, a total of 24 phyla and 379 genera were detected with predominance of three genera: *Streptococcus* (31%), *Moraxella* (30%), and *Haemophilus* (20%). The nasopharyngeal microbiota characteristics differed by 25OHD status (Table 1). Infants with lower 25OHD levels had significantly reduced richness and Shannon index than those with higher 25OHD levels (both P<0.05). While the abundance of most common genera did not differ significantly between the two groups (adjusted P>0.05, except for *Staphylococcus*), the microbiota profiles differed significantly by 25OHD status (P=0.04).

There was a significant interaction between serum total 25OHD levels and nasopharyngeal microbiota profiles on the risk of intensive care use ($P_{interaction}$=0.02), indicating heterogeneity in the microbiota-severity association. Indeed, among infants with lower 25OHD levels, *Haemophilus*-dominant microbiota profile was associated with a significantly higher risk of intensive care use (OR 3.08, 95%CI 1.31–7.25, P=0.01) compared to *Moraxella*-dominant profile (Figure 1). In contrast, there were no significant microbiota-severity associations among those with higher 25OHD levels (all P>0.20; Table E3). Similarly, in the sensitivity analysis using different cut-offs for 25OHD levels, *Haemophilus*-dominant profile was associated with a significantly higher risk of intensive care use *only* among infants with lower 25OHD levels (Tables E4 and E5).

In this multicenter prospective cohort study of 1,005 infants with bronchiolitis, we found significant associations of circulating total 25OHD levels with nasopharyngeal microbiota composition. Furthermore, there was a significant interaction between 25OHD levels and nasopharyngeal microbiota with regard to bronchiolitis severity. Specifically, the association of *Haemophilus*-dominant nasopharyngeal microbiota profile (compared to *Moraxella*-dominant profile) with higher severity was restricted to infants with lower 25OHD levels.

Previous studies have reported that both maternal and cord blood 25OHD levels are associated with infant gut microbiota.[5,6] In this study, we observed that infants with lower 25OHD levels had specific microbiota structures in the nasopharyngeal *airway* – e.g., a lower diversity and likelihood of *Moraxella*-dominant profile. Consistent with our findings, lower abundance of *Moraxella* has been reported to associate with a higher risk of ARIs in early childhood.[8] Our findings corroborate these earlier epidemiological reports, and extend them by demonstrating, for the first time, the interrelations between circulating 25OHD, airway microbiota, and clinical outcomes in infants with bronchiolitis.

The observed heterogeneity in the microbiota-bronchiolitis severity association – i.e., only lower 25OHD levels were associated with higher severity – is consistent with the emerging evidence. A recent meta-analysis of individual participant data from 25 randomized controlled trials demonstrated that protective effects of vitamin D supplementation against ARIs were seen in individuals with low baseline 25OHD levels.[9] Regardless, the mechanisms underlying the observed heterogeneity remain to be elucidated. It is possible that 25OHD contributed, through upregulation of antimicrobial peptide LL-37, to the microbiota-severity association. However, the association remained significant after adjusting for serum LL-37 levels. Alternatively, low 25OHD levels are related to up-regulation of proinflammatory cytokines and an impaired epithelial barrier, leading to enhanced inflammation and dysbiosis.[3] Furthermore, we have previously shown that lower circulating 25OHD levels associate with specific nasopharyngeal metabolomic signature – e.g., enriched proinflammatory lipids that can serve as mediators in the microbiome-host interactions in the airways.[2]

This study has potential limitations. First, we examined nasopharyngeal microbiota. Yet, studies have shown reliable correlation between upper and lower airway microbiota.[10] Serum 25OHD and nasopharyngeal microbiota were measured at a single time-point during severe illness and there was no healthy control group. However, the goal was to examine the

interrelations between circulating 25OHD, nasopharyngeal microbiota, and severity among infants hospitalized for bronchiolitis, not the development of bronchiolitis. The observed associations do not necessarily prove causality and might be explained by unmeasured confounders. However, in the current study, we rigorously adjusted for patient-level confounders, including age, prematurity, and breastfeeding, which may be associated with 25OHD levels, airway microbiota, and risks of severe ARI. Lastly, our inferences may not be generalizable to mild-to-moderate ARI. However, they remain directly relevant to the large population with severe bronchiolitis.

In summary, we found a significant interaction between serum total 25OHD levels and nasopharyngeal microbiota on bronchiolitis severity. *Haemophilus*-dominant nasopharyngeal microbiota profile was associated with an increased risk for intensive care during bronchiolitis hospitalization only in infants with lower 25OHD levels. While the causal inference remains premature, our data should facilitate further investigations into the complex interplay between early-life exposures (e.g., vitamin D), airway microbiome, and pathobiology of bronchiolitis.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

Acknowledgments:

We thank the MARC-35 study hospitals and research personnel for their ongoing dedication to bronchiolitis and asthma research (Table E1 in the Online supplement). We also thank Janice A. Espinola, MPH, Ashley F. Sullivan, MS, MPH, and Courtney N. Tierney, MPH, for their many contributions to the MARC-35 study.

Financial support: The current analysis was supported by grants UG3 OD-023253, U01 AI-087881, R01 AI-134940, and R01 AI-137091 from the National Institutes of Health (Bethesda, MD, USA). The content of this manuscript is solely the responsibility of the authors and does not necessarily represent the official views of the National Institutes of Health. Dr. Toivonen was supported by the Finnish Medical Foundation and Päivikki and Sakari Sohlberg Foundation.

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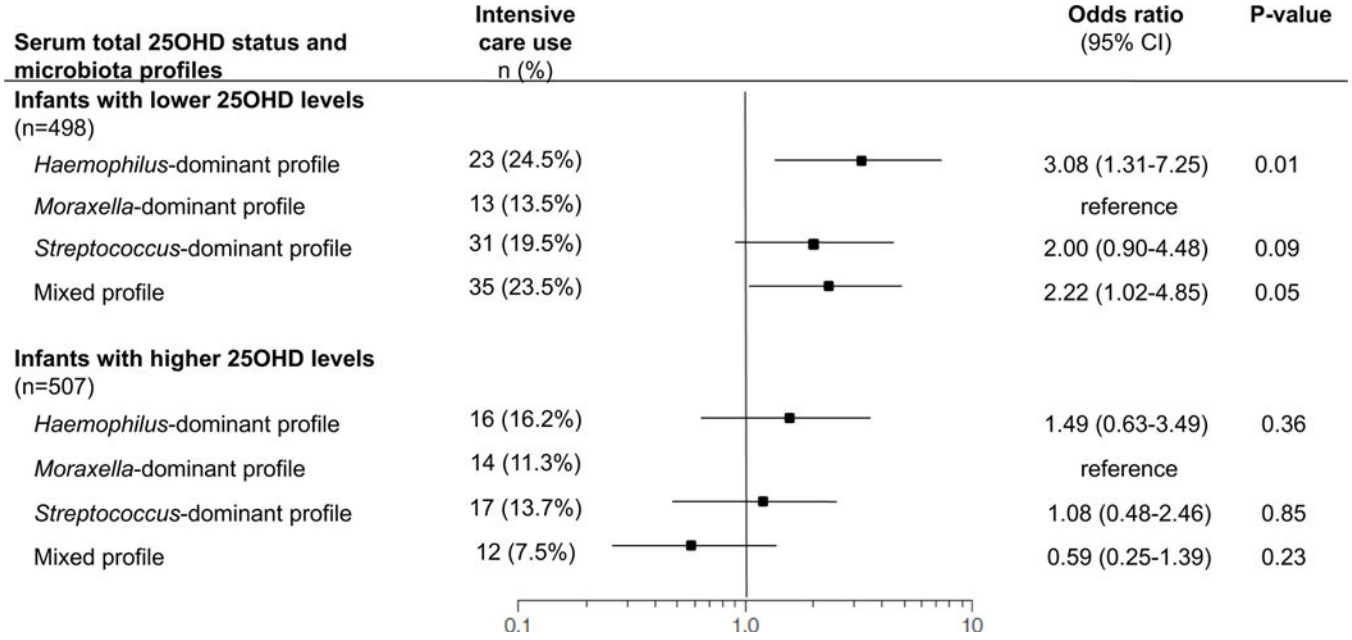

Figure 1. Associations between nasopharyngeal microbiota profiles and risk of intensive care use in infants hospitalized for bronchiolitis by serum total 25-hydroxyvitamin D (25OHD) status

Random-effects model accounting for patient clustering at the hospital-level and adjusting for 12 factors (age, sex, race/ethnicity, gestational age, siblings at home, breastfeeding, history of breathing problems, lifetime history of systemic antibiotic use, weight at hospitalization, serum LL-37 level, and virology [RSV, rhinovirus]) was constructed for each strata – i.e., infants below or above the median serum total 25OHD levels (26.5 ng/ml). *Moraxella*-dominant profile was used as the reference. Full models are presented in Table E3.

Table 1.

Nasopharyngeal microbiota of infants hospitalized for bronchiolitis by serum total 25-hydroxyvitamin D status

Characteristic	Serum total 25OHD levels		P-value
	Lower (<26.5 ng/ml), n=498	Higher (≥26.5 ng/ml), n=507	
Richness			
Number of genera, median (IQR)	15 (8–24)	17 (10–24)	0.04
Alpha-diversity			
Shannon index, median (IQR)	0.88 (0.50–1.37)	0.99 (0.62–1.45)	0.02
Relative abundance of 10 most abundant genera, mean (SD)			
Streptococcus	0.33 (0.31)	0.29 (0.28)	0.15†
Moraxella	0.28 (0.34)	0.32 (0.34)	0.15†
Haemophilus	0.20 (0.32)	0.20 (0.30)	0.98†
Prevotella	0.02 (0.06)	0.03 (0.07)	0.15†
Neisseria	0.02 (0.06)	0.03 (0.08)	0.07†
Staphylococcus	0.03 (0.11)	0.01 (0.06)	0.009†
Corynebacterium	0.02 (0.07)	0.01 (0.07)	0.43†
Alloprevotella	0.01 (0.06)	0.01 (0.04)	0.98†
Veillonella	0.01 (0.03)	0.01 (0.03)	0.20†
Gemella	0.01 (0.04)	0.01 (0.02)	0.98†
Microbiota profiles			0.04
Haemophilus-dominant profile	94 (18.9)	99 (19.5)	
Moraxella-dominant profile	96 (19.3)	124 (24.5)	
Streptococcus-dominant profile	159 (31.9)	124 (24.5)	
Mixed profile	149 (29.9)	160 (31.6)	

Abbreviations: 25OHD, 25-hydroxyvitamin D; IQR, interquartile range; SD, standard deviation

†Benjamini-Hochberg false-discovery rate adjusted P-value accounting for multiple comparisons